

5 We claim:

1. A binary hybrid mutational vector comprising an oligonucleobase targeting strand and an oligonucleobase mutator strand, wherein said targeting strand comprises:

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a) a 3' end and a 5' end; and

b) a targeting strand homologous region comprising a sequence of nucleobases homologous to a target gene sequence;

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and wherein said mutator strand comprises:

c) a 3' end and a 5' end;

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d) a complementary region comprising a sequence of nucleobases complementary to the targeting strand homologous region; and

e) a mismatch region located within the complementary region.

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2. The binary hybrid mutational vector of claim 1, wherein said targeting strand homologous region comprises at least 70% ribo-type nucleobases, and said complementary region and said mismatch region comprise at least 70% deoxyribo-type nucleobases.

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3. The binary hybrid mutational vector of claim 1, wherein said targeting strand comprises at least 70% ribo-type nucleobases and said mutator strand comprises at least 70% deoxyribo-type nucleobases.

- 5 4. The binary hybrid mutational vector of claim 2, wherein at least one ribo-type nucleobase is nuclease resistant.
5. The binary hybrid mutational vector of claim 4, wherein said nuclease-resistant ribo-type nucleobase is selected from the group consisting of 2'AX-
10 nucleosides, 2'AX-nucleotoids and 2'AR-nucleotides, wherein:
 - A is oxygen or a halogen;
 - X is hydrogen or C₁₋₆ alkyl; and
 - R is C₁₋₆ alkyl;

provided when A is a halogen, then X and R are omitted.
- 15 6. The binary hybrid mutational vector of claim 5, wherein said halogen is fluorine, chlorine or bromine.
7. The binary hybrid mutational vector of claim 2, wherein said ribo-type
20 nucleobases are ribonucleotides, and said deoxyribo-type nucleobases are deoxyribonucleotides.
8. The binary hybrid mutational vector of claim 7, wherein at least one ribonucleotide is nuclease resistant.
- 25 9. The binary hybrid mutational vector of claim 7, wherein said nuclease-resistant ribonucleotide is a 2'AR-nucleotide, wherein:
 - A is oxygen or a halogen; and
 - R is C₁₋₆ alkyl;

30 provided when A is a halogen, then R is omitted.
10. The binary hybrid mutational vector of claim 9, wherein said nuclease-resistant ribonucleotide is a 2'-O-methyl ribonucleotide.
- 35 11. The binary hybrid mutational vector of claim 3, wherein at least one ribo-

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5 type nucleobase is nuclease resistant.

12. The binary hybrid mutational vector of claim 11, wherein said nuclease-resistant ribo-type nucleobase is selected from the group consisting of 2'AX-nucleosides, 2'AX-nucleotoids and 2'AR-nucleotides, wherein

10 A is oxygen or a halogen;

X is hydrogen or C₁₋₆ alkyl; and

R is C₁₋₆ alkyl;

provided when A is a halogen, then X and R are omitted.

15 13. The binary hybrid mutational vector of claim 12, wherein said halogen is fluorine, chlorine or bromine.

14. The binary hybrid mutational vector of claim 3, wherein said ribo-type nucleobases are ribonucleotides, and said deoxyribo-type nucleobases are
20 deoxyribonucleotides.

15. The binary hybrid mutational vector of claim 14, wherein at least one ribonucleotide is nuclease resistant.

25 16. The binary hybrid mutational vector of claim 15, wherein said nuclease-resistant ribonucleotide is a 2'AR-nucleotide, wherein:

A is oxygen or a halogen; and

R is C₁₋₆ alkyl;

provided when A is a halogen, then R is omitted.

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17. The binary hybrid mutational vector of claim 16, wherein said nuclease-resistant ribonucleotide is a 2'-O-methyl ribonucleotide.

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5 18. The binary hybrid mutational vector of claim 1, wherein:

10 (a) said targeting strand further comprises a 3' hairpin formed by an intra-strand duplex region of base pairs linked by a region of contiguous unpaired bases, and the targeting strand homologous region is located adjacent to the 3' hairpin and extends in the 5' direction along the targeting strand; and

15 (b) said mutator strand further comprises a 3' hairpin formed by an intra-strand duplex region of base pairs linked by a region of contiguous unpaired bases, and the complementary region is located adjacent to the 3' hairpin and extends in the 5' direction along the mutator strand.

20 19. The binary hybrid mutational vector of claim 18 wherein the nucleobases are nucleotides.

20 20. The binary hybrid mutational vector of claim 19 wherein:

25 (a) said targeting strand further comprises a 3'-terminal ribonucleotide which is blocked from ligation and the 3' hairpin is formed by an intra-strand duplex region of 4-8 ribonucleotide base pairs linked by a region of four contiguous unpaired uracil ribonucleotides; and

30 (b) said mutator strand further comprises a 3'-terminal ribonucleotide which is blocked from ligation and the 3' hairpin is formed by an intra-strand duplex region of 4-8 deoxyribonucleotide base pairs linked by a region of four contiguous unpaired thymine deoxyribonucleotides.

21. The binary hybrid mutational vector of claim 20 wherein at least one ribonucleotide is nuclease resistant.

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5 22. The binary hybrid mutational vector of claim 21 wherein said nuclease-resistant ribonucleotide is a 2'AR-nucleotides, wherein:

A is oxygen or a halogen; and

R is C₁₋₆ alkyl;

provided when A is a halogen, then R is omitted.

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23. The binary hybrid mutational vector of claim 22, wherein said ribonucleotides are 2'-O-methyl ribonucleotides.

24. The binary hybrid mutational vector of claim 1, wherein:

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a) the mutator strand is longer than the targeting strand, and further comprises a hairpin at each end of the oligonucleobase chain having a stretch of single-stranded nucleobases disposed therebetween, wherein the stretch of single-stranded nucleobases comprises the complementary region; and

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b) the targeting strand is substantially single-stranded and the targeting strand homologous region is coextensive with the targeting strand.

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25. The binary hybrid mutational vector of claim 24, wherein said targeting strand comprises at least 70% ribo-type nucleobases and said mutator strand comprises at least 70% deoxyribo-type nucleobases.

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26. The binary hybrid mutational vector of claim 25, wherein said ribo-type nucleobases are ribonucleotides, and said deoxyribo-type nucleobases are deoxyribonucleotides.

27. The binary hybrid mutational vector of claim 26, wherein at least one ribonucleotide is nuclease resistant.

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5 28. The binary hybrid mutational vector of claim 27, wherein said nuclease-resistant ribonucleotide is a 2'AR-nucleotide, wherein:

A is oxygen or a halogen; and

R is C₁₋₆ alkyl;

provided when A is a halogen, then R is omitted.

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29. The binary hybrid mutational vector of claim 28, wherein said nuclease-resistant ribonucleotide is a 2'-O-methyl ribonucleotide.

15 30. A method of producing a binary hybrid mutational vector comprising the steps of:

20 (a) providing a separately synthesized oligonucleobase targeting strand and oligonucleobase mutator strand, wherein said targeting strand comprises:

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1) a 3' end and a 5' end; and

2) a targeting strand homologous region comprising a sequence of nucleobases homologous to a target gene sequence;

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and wherein said mutator strand comprises:

3) a 3' end and a 5' end;

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4) a complementary region comprising a sequence of nucleobases complementary to the targeting strand homologous region; and

35 5) a mismatch region located within the complementary region; and

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5 b) mixing said targeting strand and said mutator strand such that the mutator strand complementary region containing the mismatch region forms a duplex with the targeting strand homologous region.

10 31. The method of claim 30, wherein said targeting strand homologous region comprises at least 70% ribo-type nucleobases, and said mutator strand complementary and mismatch regions comprise at least 70% deoxyribo-type nucleobases.

15 32. The method of claim 30, wherein said targeting strand comprises at least 70% ribo-type nucleobases and said mutator strand comprises at least 70% deoxyribo-type nucleobases.

20 33. The method of claim 31, wherein said ribo-type nucleobases are ribonucleotides, and said deoxyribo-type nucleobases are deoxyribonucleotides.

34. The method of claim 32, wherein said ribo-type nucleobases are ribonucleotides, and said deoxyribo-type nucleobases are deoxyribonucleotides.

25 35. The method of claim 33 or 34, wherein at least one ribonucleotide is a 2'-O-methyl ribonucleotide.

36. An oligonucleobase set comprising an oligonucleobase targeting strand and a plurality of oligonucleobase mutator strands, wherein said targeting strand comprises:

30 a) a 3' end and a 5' end; and

5 b) a targeting strand homologous region comprising a sequence of nucleobases homologous to a target gene sequence;

and wherein each mutator strand comprises:

10 c) a 3' end and a 5' end;

 dd) a complementary region comprising a sequence of nucleobases complementary to the targeting strand homologous region; and

15 e) a unique mismatch region located within the complementary region.

37. The oligonucleobase set of claim 36, wherein the targeting strand is hybridized to each mutator strand to form an array of binary hybrid mutational vectors.

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38. The oligonucleobase set of claim 36 wherein the targeting strand and each mutator strand are provided in separate containers.

39. A kit comprising:

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1) the oligonucleobase set of claim 37; and

2) instructions for introducing said binary hybrid mutational vectors into cells.

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5 40. A kit comprising:

- 1) the oligonucleobase set of claim 38;
- 2) instructions for hybridizing said targeting and mutator strands to produce a binary hybrid mutational vector; and
- 3) instructions for introducing said binary hybrid mutational vector into
10 a cell.

41. A method of altering a target gene sequence, comprising the steps of:

- a) providing a cell having a target gene sequence; and
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- b) introducing the binary hybrid mutational vector of claim 1 into said cell, so that said binary hybrid mutational vector alters said target gene sequence.

20 42. The method of claim 41, wherein said targeting strand comprises at least 70% ribo-type nucleobases and said mutator strand comprises at least 70% deoxyribo-type nucleobases.

25 43. The method of claim 42, wherein said ribo-type nucleobases are ribonucleotides and said deoxyribo-type nucleobases are deoxyribonucleotides..

44. The method of claim 43, wherein said ribonucleotides are 2'-O-methyl ribonucleotides.

30 45. The method of claim 41 wherein said cell is a eukaryotic cell.

46. The method of claim 45 wherein said cell is a mammalian cell.

5 47. The method of claim 46 wherein said mammalian cell is an ovum with a pronucleus, and said binary hybrid mutational vector is introduced by direct injection into said pronucleus.

10 48. The method of claim 46 wherein said mammalian cell is an embryonic stem cell.

49. The method of claim 45 wherein said eukaryotic cell is a plant cell.

50. A method of treating a genetic disease, comprising:

15 a) providing a subject having at least one vector-repairable mutation associated with said disease;

20 b) placing a population of cells from said subject in culture, wherein said cells carry the mutation;

25 c) introducing a binary hybrid mutational vector or mixture of binary hybrid mutational vectors of claim 1 designed to correct said vector-repairable mutation into said cells to form mutation-corrected cells; and

 d) reimplanting said mutation-corrected cells into the subject.

51. The method of claim 50 wherein the subject is a human.

30 52. The method of claim 50, wherein said targeting strand comprises at least 70% ribo-type nucleobases and said mutator strand comprises at least 70% deoxyribo-type nucleobases.

5 53. The method of claim 52, wherein said ribo-type nucleobases are ribonucleotides and said deoxyribo-type nucleobases are deoxyribonucleotides.

54. The method of claim 53, wherein said ribonucleotides are 2'-O-methyl ribonucleotides.

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55. The method of claim 50 wherein said cells are selected from the group consisting of hematopoietic cells, hematopoietic stem cells, hepatocytes, hepatic reserve cells and embryonic stem cells.

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56. The method of claim 50 wherein said genetic disease is selected from the group consisting of hemoglobinopathies, beta-thalassemia, Gaucher Disease, familial hypercholesterolemia, emphysema, hemophilia and Christmas Disease.

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57. A method of selecting mutated cells from among unmutated cells comprising the steps of:

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- a) providing a population of cells to be mutagenized;
- b) transfecting said population of cells with at least one binary hybrid mutational vector of claim 1 to produce mutant cells having a selectable phenotype; and
- c) isolating said mutants having the selectable phenotype with appropriate culture conditions.

5 58. A method of constructing a transgenic mammal, comprising:

- a) obtaining a mammalian gamete;
- b) providing one or more binary hybrid mutational vectors of claim 1 for
10 alteration of a target gene sequence in the gamete;
- c) introducing said one or more binary hybrid mutational vectors into the nucleus of the gamete to produce an altered gamete and fusing the altered gamete with another cell to form an altered zygote;
- d) implanting the altered zygote into a female mammal capable of bearing the altered zygote to term; and
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- e) allowing the zygote to develop to term.

20 59. A method of constructing a transgenic mammal, comprising:

- a) obtaining a mammalian zygote;
- b) providing one or more binary hybrid mutational vectors of claim 1 for
25 alteration of a target gene sequence in the zygote;
- c) introducing said one or more binary hybrid mutational vectors into the nucleus or pronucleus of the zygote to form an altered zygote;
- d) implanting the altered zygote into a female capable of bearing the zygote to term; and
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- e) allowing the zygote to develop to term.

5 60. A method of constructing a transgenic plant, comprising:

- a) providing a plant cell or protoplast containing a target gene sequence;
- b) introducing the binary hybrid mutational vector of claim 1 into said plant cell or protoplast, so that said binary hybrid mutational vector alters said target gene sequence to form an altered plant cell or an altered protoplast; and
- c) obtaining a mature plant from the altered plant cell or the altered protoplast.

10 61. The method of claim 60 wherein said binary hybrid mutational vector is introduced by microinjection, electroporation, particle bombardment or direct oligonucleobase uptake.

15 62. The binary hybrid mutational vector of claim 1, wherein the binary hybrid mutational vector is modified for *in vivo* administration.

20 63. The binary hybrid mutational vector of claim 1 wherein the binary hybrid mutational vector is carried by a structure.

25 64. The binary hybrid mutational vector of claim 63 wherein said structure is selected from the group consisting of liposomes, micelles and microcapsules.

30 65. The binary hybrid mutational vector of claim 64 wherein said structure is modified to affect its biodistribution.

35 66. A sterile, pyrogen-free pharmaceutical composition comprising the binary hybrid mutational vector of claim 62 or 65.

5 67. A method of altering a target sequence on a target cell *in vivo*, comprising the steps of:

a) providing a subject having a target cell with a target gene sequence; and

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b) administering the binary hybrid mutational vector of claim 1 into said subject *in vivo*, so that said binary hybrid mutational vector alters the target gene sequence in the target cell.

15 68. The method of claim 67 wherein the binary hybrid mutational vector is modified for *in vivo* administration.

69. The method of claim 67 wherein the binary hybrid mutational vector is carried by a structure.

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70. The method of claim 69 wherein said structure is selected from the group consisting of liposomes, micelles and microcapsules.

25 71. The method of claim 70 wherein said structure is modified to affect its biodistribution.